De novo combination of lamivudine and adefovir versus entecavir monotherapy for the treatment of naïve HBeAg-negative chronic hepatitis B patients

Li-Chun Wang · En-Qiang Chen · Jing Cao ·
Li Liu · Li Zheng · Da-Jiang Li · Lu Xu ·
Xue-Zhong Lei · Cong liu · Hong Tang

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Abstract

Purpose Either combination treatment or monotherapy using agents with a high genetic barrier are recommended for hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB). The aim of this study was to compare effect of naïve HBeAg-negative CHB patients with either de novo combination of lamivudine (LAM) and adefovir dipivoxil (ADV) or entecavir (ETV) monotherapy.

Methods HBeAg-negative CHB patients ($n = 71$) with ALT levels between 2 and 10 times the upper normal limit and HBV DNA levels $>10^4$ copies/mL were enrolled. Patients were treated with either LAM 100 mg plus ADV 10 mg per day ($n = 31$) or ETV 0.5 mg per day ($n = 40$) for 48 weeks.

Results The average reduction in HBV DNA level compared with baseline were 5.16 ± 1.69 log in the LAM + ADV group and 5.36 ± 1.70 log in the ETV group by week 48 ($P = 0.624$). The virological response (VR) rates were 80.65 and 77.5%, the biochemical response (BR) rates were 93.55 and 90.00% at week 48 in the LAM + ADV and ETV

groups, respectively. There was no significant difference in the VR and BR between the two groups. During the 48-week treatment period, virological breakthrough and serious side effects were not noted in any patient.

Conclusions Both LAM + ADV combination therapy and ETV monotherapy are effective in naïve HBeAg-negative CHB patients, but further studies are needed to obtain long-term results.

Keywords Chronic hepatitis B · HBeAg-negative · Lamivudine · Adefovir dipivoxil · Combination treatment · Entecavir · Monotherapy

Introduction

Chronic hepatitis B HBeAg-negative patients have increasingly been garnering attention from the medical community. Existing data show that compared to HBeAg-positive patients, HBeAg-negative chronic hepatitis B (CHB) patients usually have lower levels of HBV DNA, are older, and have a higher risk for developing end-stage liver diseases, such as cirrhosis and hepatocellular carcinoma. Effective antiviral therapy is therefore warranted in HBeAg-negative CHB patients. In accordance with national guidelines for liver diseases, the loss of the hepatitis B surface antigen (HBsAg) or seroconversion of HBsAg/anti-HBs are regarded as the end point of the treatment for HBeAg-negative CHB patients [1], and in HBeAg-negative CHB patients, duration of the treatment is generally much longer compared with HBeAg-positive patients [2]. An insufficient treatment course will result in the recurrence of hepatitis [3]. One of the major concerns with extended treatment regimens, particularly when patients are treated with nucleoside/nucleotide analogs is

Li-Chun Wang and En-Qiang Chen are co-first authors.

L.-C. Wang · E.-Q. Chen · L. Liu · L. Zheng · D.-J. Li ·
L. Xu · X.-Z. Lei · C. liu · H. Tang (✉)
Centre of Infectious Diseases, West China Hospital,
Sichuan University, Chengdu, China
e-mail: htang6198@hotmail.com

L.-C. Wang · E.-Q. Chen · L. Liu · L. Xu · X.-Z. Lei · C. liu ·
H. Tang
Division of Infectious Diseases, State Key Laboratory
of Biotherapy, Sichuan University, Chengdu, China

J. Cao
Information Centre, Sichuan Provincial Hospital for Women
and Children, Chengdu, China

the increased incidence of drug resistance. As a result, methods of choosing appropriate agents for the initial treatment of HBeAg-negative CHB patients to achieve a good effect, while simultaneously reducing the occurrence of drug resistance is an important topic in clinical practice.

It is currently suggested that initial therapies involving either a combination of nucleoside/nucleotide analogs or monotherapy are both good options to prevent the development of resistance, especially, for the patients who need long-term treatment [4]; however, relevant data from forecast research on efficacy, potential side effects, or an economic evaluation for the two strategies are rare.

There are four kinds of available nucleoside/nucleotide analogs in China. Lamivudine (LAM) was the first oral agent approved for the treatment of CHB. LAM has a well-established safety and efficacy profile, but has the highest incidence of resistant mutations compared with other nucleoside/nucleotide analogs. Adefovir dipivoxil (ADV) has the characteristics of exact effect, a low drug resistance rate, and no cross resistance with other nucleoside analogs. Telbivudine has a potent effect and a relatively higher seroconversion rate and entecavir (ETV), known for its potent anti-HBV effect, has the lowest rate of resistance, but is expensive. In summary, due to different mutation sites compared to the other three nucleoside analogs, ADV was selected as a basic agent of combination therapy. As the first approved agent for CHB patients, LAM was selected due to the abundant clinical experience and lowest cost. Evidence-based medicine identified that combination therapy could reduce drug-associated resistance to ensure long-term therapy [5], especially, for LAM failure and liver transplanted patients [6, 7]. Adding ADV in LAM-resistant patients enhances the virological and biochemical responses [8], and the combination of ADV and LAM results in greater viremia reduction than ADV monotherapy [9, 10]. Thus, LAM and ADV were selected for the de novo combination treatment option.

ETV is a deoxyguanosine analog with powerful activity in inhibiting viral replication [11]. It is regarded as a high genetic barrier drug, as more than three sites for drug resistance related mutation are required [12]. Available data indicate that ETV was recommended as a first line option for long-term treatment of naïve CHB patients instead of LAM resistant patients. In naïve CHB patients, the 5-year rate of phenotypic resistance and virus breakthrough-related phenotype resistance were only 1.2 and 0.8%, respectively, in patients treated with ETV [13], but 51 and 43% in LAM resistant patients [14].

The purpose of this study was to compare the effect of naïve HBeAg-negative CHB patients treated with either LAM plus ADV or ETV monotherapy for 48 weeks. The data generated in this study were anticipated to provide

researchers and practitioners with more information regarding these two strategies in naïve HBeAg-negative CHB patients.

Patients and methods

Patients and study design

All patients in this study were outpatients of the West China Hospital of Sichuan University between October 2007 and March 2008. Patients were included in the study if they were HBsAg positive, but negative for e antigen (HBeAg) for at least 6 months prior to enrollment, had ALT levels between 2 and 10 times the upper normal level (UNL), and HBV DNA levels $>10^4$ copies/mL. All patients had never received antiviral treatment previously. Patients were excluded if they were co-infected with human immunodeficiency virus (HIV) and other hepatitis viruses or with fatty liver, alcoholic hepatitis, and/or decompensated cirrhosis.

The study was designed as a prospective case-control study. The patients were distributed semi-randomly to the combination therapy group and monotherapy group according to their medical card number with odd number in combination group and even number in monotherapy group. Baseline data were compared between two groups to ensure comparability. All patients provided written informed consent before commencing antiviral therapy. Patients in the combination therapy group were prescribed LAM 100 mg and ADV 10 mg per day while the monotherapy group received ETV 0.5 mg per day.

Serum assays

Analysis of hepatic and renal function, which include serum levels of total bilirubin (TB), ALT, albumin (ALB), blood urea nitrogen (BUN), and creatinine (Cr), was performed at baseline and again at weeks 12, 24, 36, and 48 of the treatment using an Automatic Biochemistry analyzer (Olympus AU5400, Olympus Corporation, Tokyo, Japan). Status of HBsAg, HBeAg, and antibody to HBeAg (anti-HBe) were measured by a microparticle enzyme-linked immunosorbent assay (ELISA, Santa Cruz Biotech Co., Ltd, China) at baseline and again at weeks 24 and 48 of the treatment. Serum HBV DNA was quantified by real-time polymerase chain reaction (real-time PCR) with a linear range between 1×10^3 and 5×10^7 copies/mL (DA AN Gene Co. Ltd., Guangzhou, China) at baseline and again at weeks 12, 24, 36, and 48 of the treatment. LAM-, ADV-, and ETV-associated mutations were assessed for patients with virologic breakthrough via direct sequencing.

Definitions

Virological effect (VE) was assessed based on the virological response (VR), defined as a reduction of HBV DNA levels to lower level than detection level ($<10^3$ copies/mL) as well as by the average reduction level of HBV DNA compared with baseline. The biochemical response (BR) was defined as normalization of ALT levels. Serological response (SR) was defined as disappearance of HBsAg (with or without the appearance of HBsAb).

Adverse events including emerging symptoms and abnormal laboratory tests that occurred during the treatment period were noted and critically reviewed. The VR, BR, and SR were calculated and compared between the LAM plus ADV and ETV monotherapy groups at weeks 12, 24, 36, and 48 of the treatment.

Statistical Analysis

Quantitative data were presented as the mean $\pm$ standard deviation (SD), categorical data were presented as counts and percentages, and HBV DNA levels were presented as log transformation. Data were analyzed using the SPSS software package version 13.0 (SPSS Inc., Chicago, IL, USA). Either the *t* test or Rank Sum Test was used for quantitative variables, while Pearson Chi-Square or Fisher exact tests were used for categorical variables. All tests of significance were two-tailed, and significance was defined as $P < 0.05$.

Results

Patient data

As described in Table 1, 71 patients were included in either the LAM + ADV combination group ($n = 31$) or the ETV monotherapy group ($n = 40$). In the combination group, 28 patients were male (90.32%) and mean patient age was 31 ± 6.78 years (range 19–42 years). Ten of these patients (32.26%) were diagnosed with compensated liver cirrhosis by abdominal ultrasound. In the monotherapy group, there

were 34 males (85%) and mean patient age was 29.83 ± 6.01 years (range 20–40 years). Of these, 12 patients (30%) were diagnosed with compensated liver cirrhosis.

Median level of HBV DNA in the LAM + ADV group was 1.42×10^6 copies/mL (range 1.25×10^4 – 5.0×10^7 copies/mL), mean ALT was 165.58 ± 80.58 IU/mL (range 91–392 IU/mL), and 20 patients (64.52%) were anti-HBe positive. In the ETV group, the median level of HBV DNA was 9.04×10^5 copies/mL (range 2.0×10^4 – 5.0×10^7 copies/mL), mean ALT was 140.68 ± 67.68 IU/mL (range 79–362 IU/mL), and 25 patients (62.5%) were anti-HBe positive. No significant difference in baseline characteristics existed between the two groups.

Virological effect

The average reduction in HBV DNA level in the LAM + ADV compared to baseline levels was 2.05 ± 1.77 , 4.03 ± 1.94 , 4.78 ± 1.55 , and 5.16 ± 1.69 log₁₀ copies/mL at weeks 12, 24, 36, and 48 of treatment, respectively. In the ETV group, the average reduction in HBV DNA level was 2.87 ± 1.64 , 3.98 ± 1.89 , 4.90 ± 1.57 , and 5.36 ± 1.70 log₁₀ copies/mL at weeks 12, 24, 36, and 48, respectively. As illustrated in Fig. 1, no statistically significant difference existed between the two groups ($P = 0.411$, 0.492, 0.398, and 0.624 at weeks 12, 24, 36, and 48, respectively).

Of the 31 patients in the LAM + ADV group, there were 6, 13, 20, and 25 patients who achieved undetectable HBV DNA by weeks 12, 24, 36, and 48, respectively. The corresponding VRs were 19.35, 41.94, 64.52, and 80.65%. Of the 40 patients in the ETV group, 10, 18, 25, and 31 patients achieved undetectable HBV DNA by weeks 12, 24, 36, and 48, respectively. The corresponding VRs were 33.33, 45.00, 62.5, and 77.50%. As illustrated in Fig. 2a, by week 12, the VR in the ETV group was higher than in the LAM + ADV group, but the difference between two groups was not statistically significant ($P = 0.946$). Similarly, by weeks 24, 36, and 48, the difference in VR between the two groups was not statistically significant ($P = 0.796$, 0.874, and 0.994).

Table 1 Baseline characteristics of the patients included in the LAM + ADV combination therapy and ETV monotherapy treatment groups

Group	LAM + ADV	ETV	<i>P</i> value
Patients enrolled	31	40	
Male gender	28 (90.32)	34 (85%)	0.757
Age (years)	31 ± 6.78	29.83 ± 6.01	0.160
HBV DNA (media)	1.42×10^6	9.04×10^5	0.651
Alanine aminotransferase(ALT)	165.58 ± 80.58	140.68 ± 67.68	0.162
HBeAb-positive	20 (64.52%)	25 (62.50%)	0.861
Compensated cirrhosis	10 (32.26%)	12 (30.0%)	0.365

Data are presented as either number (percent) or mean $\pm$ SD

Fig. 1 Serum HBV DNA levels in naïve HBeAg-negative CHB patients treated with LAM + ADV combination therapy and ETV monotherapy

Fig. 2 Response rates of naïve HBeAg-negative CHB patients treated with LAM + ADV combination therapy and ETV monotherapy: **a** virological response, **b** biochemical response

Biochemical response

Of the 31 patients receiving LAM + ADV, there were 4, 19, 27, and 29 patients who achieved ALT normalization by weeks 12, 24, 36, and 48, respectively. Corresponding BR rates were 12.90, 61.29, 87.1, and 93.55%. Of the 40 patients receiving ETV, there were 11, 31, 35, and 36 patients who achieved ALT normalization by weeks 12, 24,

36, and 48, respectively. Corresponding BR rates were 27.50, 77.50, 87.5, and 90.00%. As illustrated in Fig. 2b, by weeks 12, 24, and 36, the BR rates of ETV were higher than those in the LAM + ADV group but the BR rates were lower in the ETV group than the LAM + ADV group by week 48; however, none of these differences were statistically significant ($P = 0.135, 0.138, 0.276, 0.594$, at weeks 12, 24, 36, and 48, respectively).

Serological response

No patient in either of the two treatment groups had loss of HBsAg and/or HBsAg/anti-HBs serological conversion by week 48.

Virological breakthrough and drug resistance

During the 48-week treatment period, virological breakthrough did not occur in any of the 71 patients included in this study. That is, LAM-, ADV-, or ETV-associated mutations were not detected.

Adverse events

All patients in this study tolerated the LAM + ADV and ETV well. Adverse events in the LAM + ADV group were noted in only one patient by week 24. This patient's BUN increased to 7.87 $\mu\text{mol/L}$ without a concomitant increase in Cr levels. The patient was monitored closely but did not receive any additional treatment. The BUN of this patient was 8.03 $\mu\text{mol/L}$ at week 28, and 7.56 $\mu\text{mol/L}$ at week 32. Levels were normalized by week 36 and remained within normal limits for the duration of the study period. In the ETV group, there was one patient with a baseline platelet count of $93 \times 10^9/\text{mL}$ which subsequently decreased to $76 \times 10^9/\text{mL}$ by week 12. Without special treatment, platelet (PLT) count increased to 91×10^9 per mL by week 18. For the duration of the study period, PLT count fluctuated between 89 and 95×10^9 per mL. No bleeding was observed in this patient.

Discussion

A consensus on the benefits of antiviral therapy for CHB patients has been reached and reported [15]. The rapid development of nucleoside/nucleotide analogs has played an important role in antiviral therapy, especially, for HBeAg-negative patients [16]. Five-year clinical data found that long-term antiviral therapy with nucleoside drugs significantly inhibits viral replication and prevents the occurrence of decompensated cirrhosis. In addition, nucleoside analogs are convenient to take and associated with good safety profiles. As such, nucleoside/nucleotide analogs are widely prescribed in clinical practice.

Due to high replication rates of HBV, lack of proof reading or editing activity for reverse transcriptase, and continued existence of replication template cccDNA, HBV mutations can and do develop, resulting in drug resistance during long-term treatment. Drug resistance is one of the most important influencing factors limiting long-term nucleoside treatment for CHB patients [17]. When

virological breakthrough or drug resistance occurs, rescue therapy is recommended; however, rescue therapy not only decreases the effect of antiviral treatment, but also is unable to eliminate the drug-resistant strain(s). Multi-drug resistance can occur. Rescue therapy usually prolongs the course of treatment, increases treatment-associated costs significantly, and makes additional therapy more challenging.

It has been reported that the duration of treatment of HBeAg-negative patients was generally much longer than that of HBeAg-positive patients, accompanied with increased incidence of drug resistance. It is, therefore, important to prevent drug resistance in nucleoside-naïve patients, particularly for HBeAg-negative patients and patients with cirrhosis, who require long-term treatment [18]. According to the 2009 EASL guidelines, initial combination therapy or use of agents with a high genetic barrier are recommended in patients with a high risk of developing drug resistance and potentially life-threatening associated diseases (i.e., cirrhosis). Based on this principle, the best option for naïve CHB patients who are HBeAg-negative or have serious liver disease, such as cirrhosis is either combination treatment or monotherapy with high genetic barrier drugs to reduce the occurrence of HBV resistance. Nonetheless, it remains unclear what agents should be combined, duration of therapy, and when to change or stop treatment [19]. The potential effect, safety, rate of drug resistance, and route of administration should be considered for any new strategy.

All cases included in this study were HBeAg-negative CHB patients, including those with liver cirrhosis in whom initial combination therapy or monotherapy with high genetic barrier is suitable. In this study, the efficiency of ETV initial monotherapy compared with the combination of LAM and ADV in HBeAg-negative CHB patients was investigated. The study was a prospective case-control study. Although standard randomization was not applied, semi-random method was adopted and baseline data were compared between two groups to ensure comparability.

The results of this study showed that both therapy strategies inhibit viral replication and improve liver function. This was achieved faster with ETV than the combination of LAM and ADV as the VR of ETV was higher than that of the LAM + ADV combination by week 12, but the difference was not statistically significant. At weeks 24 and 48 of treatment, the difference in VR between ETV monotherapy and the LAM + ADV combination therapy was not significant. These data suggested that the power of ETV monotherapy and LAM + ADV combination therapy were similar in inhibiting viral replication. Similar results were observed for the BR.

Likely because of the limited treatment period, no patient achieved loss of the HBsAg or HBsAg/Anti-HBs serological conversion in this study. At the same time, no virological breakthrough or drug resistance occurred in either of the two treatment groups by week 48 of the study.

Both treatment strategies were well tolerated, with a low incidence of adverse reactions.

With the gradual extension of the treatment course, not only effect but also cost should be evaluated. In this study, the cost-effectiveness analysis was not done because of relative short-term therapy. So it is valuable to carry on further long-term study to compare cost-effectiveness ratio of the two different treatment strategies.

Previous reports cited that the virus response of LAM after 48 weeks of treatment was only 43.8% for HBeAg-negative CHB patients [20] and the cumulative rates of genotypic resistance (GR) after 1 year was as high as 15% [21]. Viral suppression occurred in HBeAg-negative patients receiving ADV for 48 weeks was 63% with no amino acid substitutions within the HBV DNA polymerase associated with phenotypic resistance [22]. These data show that for HBeAg-negative CHB patients, de novo combination treatment of LAM + ADV for 48 weeks increases the effect of viral suppression compared with LAM and ADV monotherapy and reduces the occurrence of drug resistance significantly compared with LAM monotherapy. This strategy was worthy for further clinical application in countries, where ETV is not available or very expensive.

As mentioned above, both LAM + ADV combination therapy and ETV monotherapy effectively inhibit viral replication and improve liver function in naïve HBeAg-negative CHB patients during a 48-week treatment period. Neither treatment strategy had virus breakthrough nor did have good safety. Based on these results, either LAM + ADV combination therapy or ETV monotherapy is appropriate for the treatment of naïve HBeAg-negative CHB patients for 48 weeks. However, as we know, the primary end point for HBeAg-negative CHB is HBsAg loss, so the follow-up of 48 weeks is too short. In fact, all our patients are still receiving antiviral therapy and further study is necessary to confirm current results.

The major limitations of the study include the relatively small number of patients ($n = 71$), the relatively short observation period (48 weeks) which influenced evaluation of long-term efficacy, accumulation of drug resistance, identification of adverse reactions, and economical evaluation. It is necessary to be concerned with prolonging the course of treatment in the future.

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